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怒茶素——怒江山茶的一个新黄酮甙

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摘要：用葡聚糖凝胶和树脂层析技术从云南省昆明地区产怒江山茶（*Camellia saluenensis* Stapf ex Bean）的鲜叶中分离到 11 个酚类化合物，其中 10 个分别 鉴定为已知的槲皮素类黄酮化合物及原花色素类化合物。另 1 个为新的黄酮甙，经光谱与化学方法测定，其化学结构为槲皮素－3－O－β－D－木吡喃糖基(1→2)－α－L－鼠李吡喃糖基(1→6)－β－D－葡萄吡喃糖甙，命名为怒茶素。

关键词：山茶科；怒江山茶；酚类化合物；黄酮甙；怒茶素
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Saluenin, a New Flavonol Glycoside from *Camellia saluenensis*

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Abstract: By means of column chromatography of sephadex and macroporous resin, a new flavonol glycoside, saluenin, was isolated from the fresh leaves of *Camellia saluenensis* together with ten known phenolic compounds. The structure of saluenin was identified as quercetin 3－O－β－D－xylopyranosyl(1→2)－α－L－rhamnopyranosyl(1→6)－β－D－glucopyranoside by spectral data and chemical methods.

Key words: Theaceae; *Camellia saluenensis*; Phenoloids; Flavonoid glycoside; Saluenin

Camellia saluenensis Stapf ex Bean is an endemic species to Yunnan Province of China. This plant is well known as a field ornamental because of its big and beautiful flowers. In the folk medicine, the leaves are always used as antipyretic and diuretic by minority people. As a part of our phytochemical and chemotaxonomic studies on Theaceae (Zhang *et al*, 1995), this paper deals with the isolation and structure elucidation of phenolic constituents of the leaves of *C. saluenensis*.

Results and Discussion

The acetone extract of fresh leaves of *C. saluenensis* was repeatedly chromatographed on Diaion Gel, Sephadex LH－20, MCI Gel CHP 20P and TSK Gel columns to yield eleven compounds (**1**～**11**) Among them, **2**～**11** are known phenolic compounds and were identified as quercetin (**2**), quercetin 3－O－β－glucopyranoside (**3**), quercetin 3－O－β－galactopyranoside(**4**), quercetin 3－O－α－L－rhamnopyranoside(**5**), quercetin 3－O－α－L－arabinofuranoside (**6**), rutin (**7**) (Markham *et al*, 1978), (－)－epicatechin (**8**),

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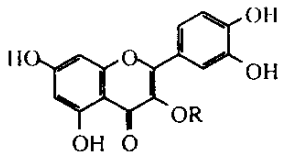
procyanidin B－2 (**9**), procyanidin C－1 (**10**) (Zhang *et al*, 1994) and 3'－O－galloylprocyanidin B－2 (**11**) (Nonaka *et al*, 1983), respectively, by their physical and spectral data. Compound **1** is a new natural product.

Compound **1** was obtained as green－yellow powder. Negative FAB mass spectrum give a quasi molecular ion peak at m/z 741 $[M(C_{32}H_{38}O_{20})-H]^-$ and fragment ions at 609 [741－pentose]$^-$ and 463 [609－deoxyl hexose]$^-$. The flavonoid skeleton of compound 1 was indicated by the proton signal of C－5 hydroxyl group at δ 12.57 (1H, s) in ^{1}H NMR spectrum and the carbonyl signal at δ 177.71 in ^{13}C NMR spectrum. The presence of three carbon signals at δ 99.02 (C－6), 94.36 (C－8) and 133.61 (C－3), two proton singals of A－ring at δ 6.35 (1H, s, H－6) and 6.17 (1H, s, H－8), as well as proton signals of 3,4－substituted B－ring at δ 7.53 (1H, s), δ 7.51 (1H, d, J＝8.4Hz) and δ 6.62 (1H, d, J＝8.4Hz), suggested that the aglycone moiety was found to be coincident with those of quercetin (**2**). Acidic hydrolysis of compound 1 gave 2 and 7. Three anomeric carbon signals of sugar moiety appeared at δ 105.53, δ 101.63 and δ 101.28 while three anomeric protons at δ 5.29 (1H, d, J＝7.2Hz), δ 4.26 (1H, s) and δ 4.21 (1H, d, J＝7.2Hz). Compared with 7, compound 1 showed a set of additional signals corresponding to a β－D－xylopyranosyl unit in the ^{13}C NMR spectrum. It was also observed that the glycosylation shift effect in a downfield shift (10.76 ppm) at the C－2 position of α－L－rhamnopyranosyl unit. It indicated that the interglycosyl linkage of terminal β－D－xylopyranosyl unit should be located on the C－2 position of α－L－rhamnopyranosyl unit. All these were proved by HMBC experiment. The anomeric proton (δ 5.29) of β－D－glucopyranosyl unit correlated with C－3 (δ 133.61) of the alycone, the anomeric proton (δ 4.26) of α－L－rhamnopyranosyl unit correlated with C－6 (δ 67.87) of β－D－glucopyranosy unit and the anomeric proton (δ 4.21, d, J＝7.2Hz, 1H) of β－D－xylopyranosyl unit correlated with C－2 (δ 81.28) of α－L－rhamnopyranosyl unit. From the above evidences, the structure of compound 1 was elucidated as quercetin 3－O－β－D－xylopyranosyl (1→2)－α－L－rhamnopyranosyl (1→6)－β－D－glucopyranoside, given the trivial name as saluenin.

It is noticed that phenolic constituents of fresh leaves of *C. saluenensis* could be divided into two types, flavonoids and procyanidins. The content of quercetin (**2**) and its derivatives is higher than that of procyanidins. It will be a significant chemical marker for the chemotaxonomy of this genus.

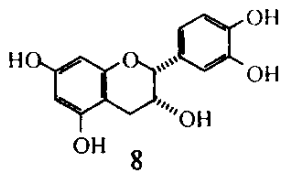
Experimental

General　Mps were determined on a Kofler hot stage apparatus and corrected by authentic sample of caffeine (237 ℃). Optical rotations were measured with SP－EA－300 apparatus. UV and IR spectra were recorded on Shimadzu UV－210A and IR－450 spectrophotometers, in MeOH and KBr pellets, respectively. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AM－400 NMR spectrometer and HMBC NMR spectra on a Bruker AM－500 NMR spectrometer in DMSO－d_6 and CD_3OD using TMS as internal standards. FAB and EI mass spectra were obtained on a VG Autospec mass spectrometer. CC was carried out on Diaion Gel, Sephadex LH－20, MCI Gel CHP 20P and TSK Gel. TLC was conducted on precoated silica gel plates. Spots were detected by spraying with $FeCl_3$ and H_2SO_4.



R

1 — Glc(6-1)Rha(2-1)Xyl
2 — H
3 — Glc
4 — Gal
5 — Rha
6 — Ara(f)
7 — Glc(6-1)Rha



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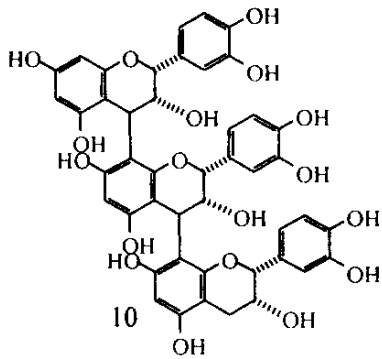

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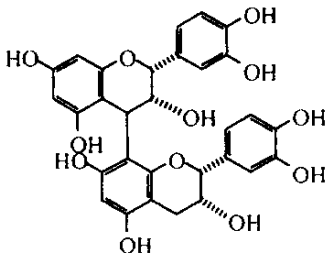

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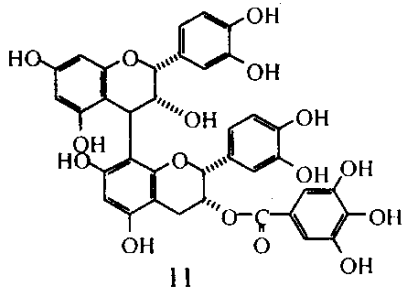

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Extraction and isolation

Plants material (*Camellia saluenensis* Stapf ex Bean) was collected in Novenmber 1996 in Kunming, Yunnan Province and identified by Prof. L. F. Xia, who is a *Camellia* expert at the Kunming Botanical Garden of the institute. The fresh leaves (1.6 kg) were extracted with 80% acetone at room temperature. The acetone extraction was concentrated under reduced pressure and diluted with water. The aqueous solution was subjected to a column contain Diaion gel and eluted successively with H_2O and MeOH, respectively. The MeOH fractions were combined, evaporated and then the residue was chromatographed over Sephadex LH－20 and eluted with ethanol. Fractions 1～7 were obtained.

Fraction 1 was chromatographed on Sephadex LH－20 column with 60% MeOH to give compound 1 (80mg).

Fraction 2 was condensed to small volume to obtain a crystal. After recrystalization, compound 8 (70mg) was obtained.

Fraction 3 was chromatographed over Sephadex LH－20 column with 60% MeOH to afford a crystal, compound 3 (500mg). The mother liquor was chromatographed by TSK gel column with 50% MeOH to afford compound 4 (200mg).

Fraction 4 was subjected to repeatedly chromatograph on Sephadex LH－20 column and TSK gel column eluted with 50%～60% MeOH to afford compound 9(200mg), 10(100mg) and 11(80mg).

Fraction 5 was chromatographed on the column of MCI Gel CHP 20P and TSK Gel eluted

with 50% MeOH to afford compound 6 (15mg).

Fraction 6 was over a column chromatography of Sephadex LH－20 eluted with 60% MeOH to afford compound 5 (100mg) and 7 (60mg).

Fraction 7 was filtered and compound 2 (500mg) was obtained.

Saluenin (1). Green yellow powder. Negative FAB－MS, m/z(%): 741[M($C_{32}H_{38}O_{20}$)－H]$^-$(100), 609[M－Xyl－H]$^-$(5), 463[609－Rha]$^-$(8). $[\alpha]_D^{25.5}$ + 63.99°(MeOH, c 0.0029). IRν_{max} cm^{-1}: 3392, 2924, 2364, 1657, 1608, 1508, 1449, 1361, 1304, 1204, 1171, 1044, 996. UVλ_{max}(nm)(MeOH): 206.5, 257.2, 261.0, 288.0, 358.5. ^{13}C and ^{1}H NMR see Table 1 and 2.

Acid hydrolysis of saluenin (1) Compound 1 (30mg) was heated in 10 mL 1 mol/L HCl at 60 ℃ for 15 min, the hydrolyzed solution was chromatographed over sephadex LH－20 eluted with water and 50% MeOH and compound 2 (8mg) and 7 (7mg) were obtained.

Quercetin (2). pale yellow powder. Negative FAB－MS, m/z(%): 301[M($C_{15}H_{10}O_7$)－H]$^-$(100%). IR ν_{max} cm^{-1}: 3324, 1665, 1611, 1562, 1522, 1452, 1407, 1382, 1319, 1261, 1214, 1199, 1170, 1132, 1092, 1014, 941. UVλ_{max}nm(MeOH): 207.5, 284, 370.5. ^{13}C and ^{1}H NMR see Table 1 and 2.

Table 1　^{13}C NMR data of compounds 1～7(in DMSO－d_6)

carbon	1	2	3	4	5	6	7
2	157.04	146.79	156.68	156.22	156.42	156.31	156.48
3	133.61	135.65	133.70	133.49	134.25	133.35	133.27
4	177.71	175.78	177.74	177.41	177.72	177.65	177.28
5	161.54	160.67	161.46	161.14	161.27	161.17	161.13
6	99.02	98.13	99.06	98.58	98.90	96.68	98.57
7	164.47	163.62	164.38	164.04	164.16	164.37	163.97
8	94.36	93.30	93.94	93.40	93.81	93.54	93.46
9	156.86	156.10	156.68	156.22	157.25	156.84	156.33
10	104.31	102.97	104.31	103.86	104.20	103.86	103.9
1'	121.65	121.92	121.50	121.06	120.74	120.93	121.48
2'	116.20	115.03	115.57	115.11	115.43	115.50	115.13
3'	145.02	145.00	145.03	144.71	145.17	145.05	144.64
4'	148.71	147.64	148.70	148.35	148.40	148.45	148.29
5'	115.50	115.55	116.51	115.59	115.66	115.50	116.20
6'	121.55	119.94	121.93	121.85	121.08	121.63	121.13
sugar	Glc Rha Xyl		Glc	Gal	Rha	Ara	Glc Rha
1	101.63 101.28 105.53		101.35	101.69	101.82	107.84	101.16 101.00
2	74.12 81.28 74.37		74.41	71.16	70.38	82.06	74.01 70.52
3	76.36 69.77 76.75		76.75	73.17	70.53	76.98	76.55 69.97
4	70.14 71.05 70.58		70.19	67.85	71.25	85.20	70.28 71.83
5	76.98 68.19 65.79		77.57	75.73	70.02	60.69	75.85 68.10
6	67.87 17.84		61.24	60.06	17.43		66.90 17.57

Quercetin 3－O－β－glucopyranoside(3). Negative FAB－MS, m/z(%): 463[M($C_{21}H_{20}O_{12}$)－H]$^-$(100%). IRν_{max} cm^{-1}: 3368, 1659, 1607, 1565, 1499, 1446, 1363, 1305, 1274, 1200, 1170, 1114, 1081, 1062, 1013, 998, 936. UVλ_{max}nm(MeOH): 207.5, 257,

356.5. ^{13}C and ^{1}H NMR see Table 1 and 2.

Quercetin 3－O－β－galactopyranoside(4). Negative FAB－MS, m/z(%)：463[M ($C_{21}H_{20}O_{12}$)－H]$^-$(100%). IR ν_{max} cm^{-1}：3318, 1658, 1608, 1565, 1503, 1453, 1367, 1260, 1209, 1174, 1126, 1091, 1051, 1021, 998, 941, 867, 816. UVλ_{max} nm (MeOH)：206, 256.5, 297, 360.5. ^{13}C and ^{1}H NMR see Table 1 and 2.

Quercetin 3－O－β－L－rhamnopyranoside(5). Negative FAB－MS, m/z(%)：447[M ($C_{21}H_{20}O_{11}$)－H]$^-$(100%). IR ν_{max} cm^{-1}：3289, 1658, 1605, 1574, 1501, 1455, 1381, 1360, 1303, 1272, 1250, 1202, 1169, 1110, 1070, 1006, 998, 964, 918, 882. UVλ_{max}nm (MeOH)：206.5, 255, 351. ^{13}C and ^{1}H NMR see Table 1 and 2.

Table 2 ^{1}H NMR data of compounds 1～7(in DMSO－d_6)

proton	1	2	3	4	5	6	7
6	6.35, s	6.40, d, J=1.6Hz	6.41,d, J=1.9Hz	6.52, s	6.38 s	6.39,d, J=1.2Hz	6.52,d, J=2.0Hz
8	6.17, s	6.17,d, J=1.6Hz	6.18,d, J=1.5Hz	6.19,d, J=2.0Hz	6.19,s	6.19,d J=1.2Hz	6.18,d, J=2.0Hz
2'	7.53, s	7.66,d, J=1.2Hz		7.51,d, J=2.4Hz	7.28, s	7.46,d, J=1.6Hz	7.52, s
5'	6.62,d J=8.4Hz	6.67,d, J=8.4Hz		6.80,d, J=8.8Hz	6.84,d, J=8.0Hz	6.84,d, J=8.4Hz	6.83,d, J=8.4Hz
6'	7.51,d, J=8.4Hz	7.53,dd, J=8.8,1.2Hz		7.66,dd J=8.4,2.0Hz	7.23,d, J=8.0Hz	7.54,dd, J=8.4,1.6Hz	7.53,dd, J=8.6,2.0Hz
C5－OH	12.57, s	12.49, s	12.60, s	12.62, s	12.64, s	12.62, s	12.62, s
Anomeric H							
Glc	5.29,d, J=7.2Hz		5.41,d, J=7.4Hz				5.34,d, J=8.0Hz
Rha	4.26,s				5.23,s		4.36,s
Xyl	4.21,d, J=7.2Hz						
Gal				5.34,d, J=8.0Hz			
Ara						5.51,s	

Quercetin 3－O－α－L－arabinofuranosid(6). Negative FAB－MS, m/z(%)：433[M ($C_{20}H_{18}O_{11}$)－H]$^-$(100%). IR ν_{max} cm^{-1}：3352, 1658, 1603, 1563, 1495, 1456, 1360, 1295, 1235, 1202, 1061, 1014, 938, 878, 802. UVλ_{max}nm(MeOH)：207, 256, 356. ^{13}C and ^{1}H NMR see Table 1 and 2.

Rutin(7) Negative FAB－MS, m/z(%)：609[M($C_{27}H_{30}O_{16}$)－H]$^-$(100%). IRν_{max} cm^{-1}：3422, 1656, 1604, 1506, 1456, 1363, 1297, 1204, 1169, 1122, 1090, 1064, 1015, 880, 809. UVλ_{max}nm (MeOH) 206, 256.5, 288, 356. ^{13}C and ^{1}H NMR see Table 1 and 2.

(－)－Epicatechin (8). White amorphous powder. Negative FAB－MS, m/z(%)：289 [M ($C_{15}H_{14}O_6$)－H]$^-$(100%). $[\alpha]_D^{17}+75°$ (c 0.3800, MeOH). IRν_{max} cm^{-1}：3392, 1608, 1510, 1440, 1365, 1285, 1202, 1160, 1110, 1060, 810. UVλ_{max}nm(MeOH)：208, 225, 280.5, 284. ^{1}H NMR(CD_3OD)：δ4.80(1H, s, H－2), 4.16(1H, m, H－3), 2.79(1H,

dd, J = 16.6, 4.6Hz, H-4A), 2.64(1H, dd, J = 16.7, 3.5Hz, H-4B), 5.87(1H, d, J = 2.2Hz, H-6), 5.98(1H, d, J = 2.2Hz, H-8), 6.98(1H, 1H, d, J = 1.4Hz, H-2'), 6.77(2H, m, H-5', 6'). ^{13}C NMR (CD3OD) δ79.1(C-2), 66.6(C-3), 28.5(C-4), 157.1(C-5), 96.2(C-6), 157.2(C-7), 95.4(C-8), 156.7(C-9), 99.6(C-10), 131.1(C-1'), 115.5(C-2'), 145.0(C-3'), 145.1(C-4'), 115.1(C-5'), 119.1(C-6').

Procyanidin B-2 (9). White amorphous powder. Negative FAB-MS, m/z(%): 577 [M ($C_{30}H_{26}O_{12}$) - H]$^-$ (100). $[\alpha]_D^{17}$ + 47.11° (c 0.0033, MeOH). IRν_{max} cm^{-1}: 3392, 1612, 1522, 1446, 1360, 1285, 1202, 1155, 1110, 1063, 823. UVλ_{max}nm(MeOH): 207, 281. ^{13}C NMR (CD_3OD): ring-A and C δ76.3(C-2), 78.7(C-2'), 72.4(C-3), 65.8(C-3'), 36.3(C-4), 28.8(C-4'), 157.3, 156.9, 155.1(C-5, 5', 7, 7', 9, 9'), 95.9, 95.4(C-6, 6', 8, 8'), 96.9(C-10), 100.1(C-10'); ring-B: 131.8(C-1), 115.6(C-2), 144.9(C-3), 144.7(C-4), 115.5(C-5), 119.0(C-6), 131.3(C-1'), 115.0(C-2'), 144.5(C-3'), 144.7(C-4'), 114.7(C-5'), 106.8(C-6').

Procyanidin C-1 (10). Brown powder. Negative FAB-MS, m/z(%): 865[M ($C_{45}H_{38}O_{18}$)$^-$ H] - (100). $[\alpha]_D^{17}$ + 70.66° (c 0.0111, MeOH). IRν_{max} cm^{-1}: 3341, 1604, 1521, 1444, 1360, 1284, 1201, 1148, 1099, 1059, 995, 972, 855. UVλ_{max} nm (MeOH): 209.5, 280.5. ^{13}C NMR (CD_3OD): ring-A and C δ79.72 (C-2), 77.11(C-2', 2''), 66.97(C-3), 73.39, 72.84(C-3', 3''), 29.69(C-4), 37.43(C-4', 4''), 154.43, 156.46, 156.86, 157.26, 157.80, 158.34(C-5, 5', 5'', 7, 7', 7'', 9, 9', 9''), 96.66, 96.31(C-6, 6', 6'', 8, 8', 8''), 97.54, 100.76(C-10, 10', 10''); ring-B: 132.67, 132.09(C-1, 1', 1''), 115.16 (C-2, 2', 2''), 115.34, 116.08(C-5, 5', 5''), 145.43, 145.54, 145.67, 145.76, 145.91(C-3, 3', 3'', 4, 4', 4''), 119.19, 107.64(C-6, 6', 6'').

Procyanidin B-2 3'-O-gallate (11). Off-white powder. Negative FAB-MS, m/z (%): 729 [M($C_{37}H_{30}O_{16}$) - H]$^-$ (100). $[\alpha]_D^{17}$ - 38.37° (c 0.0043, MeOH). IRν_{max} cm^{-1}: 3368, 1692, 1611, 1522, 1450, 1360, 1284, 1231, 1150, 1097, 1062, 1032, 872. UVλ_{max} nm (MeOH): 207, 280. ^{13}C NMR (CD_3OD): ring-A and C δ75.04(C-2), 79.92(C-2'), 76.14(C-3), 67.14(C-3'), 34.77(C-4), 29.43(C-4'), 157.54, 157.31, 156.51, 156.12(C-5, 5', 7, 7', 9, 9'), 96.45(C-6), 96.11(C-6'), 97.24(C-8), 107.25(C-8'), 102.67(C-10), 100.77(C-10'); ring B: 131.93, 131.82(C-1,1'), 116.00, 115.84, 115.20(C-2, 2', 5, 5'), 145.76, 145.65, 145.39, 145.20(C-3, 3', 4, 4'), 119.52, 119.79(C-6, 6'); Galloyl group: 121.61(C-1), 110.40(C-2, 6), 146.07(C-3, 5), 139.58(C-4), 167.20(COO).

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